doi:10.1016/j.phrp.2011.11.049
pISSN 2210-9099 eISSN 2233-6052

BRIEF REPORT

A Contribution of MdfA to Resistance to Fluoroquinolones in *Shigella flexneri*

Jun-Young Kim, Se-Mi Jeon, Hyungjun Kim, Mi-Sun Park, Seong-Han Kim*

Division of Enteric Bacterial Infections, Korea National Institute of Health, Osong, Korea.

Received: August 18, 2011
Revised: September 26, 2011
Accepted: October 27, 2011

KEYWORDS:
efflux pump,
fluoroquinolones resistance,
MdfA,
Shigella flexneri

Abstract

In this study, we measured the drug resistance conferred by *mdfA* mutations in two *Shigella flexneri* strains. A mutant in *mdfA* genes was constructed by polymerase chain reaction–based, one-step inactivation of chromosomal genes. The antimicrobial susceptibility of parent and mutant strains to fluoroquinolones was determined by minimal inhibitory concentration (MICs). The △*mdfA* mutants were somewhat more susceptible to fluoroquinolones than the parent strains. The low level changes in MICs of the △*mdfA* mutants suggest that *mdfA* contributed the fluoroquinolone resistance in *S flexneri*. This finding found that the increased expression level of an MdfA efflux pump mediated fluoroquinolone resistance, but it is not likely a major effecter of higher resistance levels.

1. Introduction

In the recent paper in the resistance to fluoroquinolones by the combination of target site mutations and enhanced expression of genes for efflux pumps in *Shigella flexneri* [1], we presented that MdfA could be related to fluoroquinolone resistance.

The most common example of an major facilitator superfamily (MFS) antibiotic efflux system in gram-negative bacteria is that encoded by the various *tet* genes associated with tetracycline efflux and resistance [2]. Members of this family of efflux fluoroquinolones are, by contrast, rare in gram-negative bacteria and include only the MdfA transporter of *Escherichia coli* [3,4]. Therefore, we would like to report the additional evidences that the MdfA is related to fluoroquinolone resistance in *S flexneri*, even though MdfA does appear to be a more effective pump for nonantibiotics. However, the increased expression level of the MdfA efflux pump mediating fluoroquinolone resistance was first confirmed in the *Shigella* species strains in this study [1,2]. This study demonstrated that resistance to fluoroquinolone is due to efflux by the MdfA system in the *Shigella* species. To determine the molecular basis of efflux in the *Shigella* species, a deletion mutation in *mdfA* was constructed.

*Corresponding author.
E-mail: kkingsh@chollian.net

Table 1. MICs of *S flexneri* SF021787 and SF021895 isolates

	Substitution in *gyrA* and *parC* QRDR		MIC(μg/ml)			
Strains	*gyrA*	*parC*	CIP	CIP + CCCP	NOR	NOR + CCCP
SF021787	S83L	S80I, R91Q	16	0.125	32	0.25
SF021787 △*mdfA*::*kan*	S83L	S80I, R91Q	12		4	
SF021895	S83L, D87G	S80I, R91Q	16	0.125	8	0.25
SF021895 △*mdfA*::*kan*	S83L, D87G	S80I, R91Q	12		4	

2. Methods

Deletion of the *mdfA* gene was performed by the method described by Datsenko and Wanner [5]. The kanamycin resistance gene *kan* flanked by flippase (FLP) recognition target sites was amplified by a standard polymerase chain reaction (PCR) with the templated plasmid pKD4 and hybrid primers. These primers, P1MdfA (AGCTGCGCTTTATTAAACTCTG CGCGATTA TTATTGGCGAAGAAATTGCGTGTA GGCTGGAGCTGCTTC) and P2MdfA (TCACCATT AATTCGAGAATGCCTGATCGCACAAATCAATCA GGCATTTTTATGGGAATTAGCCATGGTCC), were synthesized with 20 nucleotides (nt) of priming sites 1 and 2 of pKD4 and with 50 nt of the 5′ and 3′ ends of the *mdfA* gene. The 1.6 kb PCR fragment was purified and electroporated into *S flexneri* isolates, 021787 and 021895, into which the red recombinase expression plasmid pKD46 was introduced. Transformants were selected at 37°C on Luria−Bertani (LB) agar medium containing kanamycin at 50 ug/ml. Homologous recombination between the genomic DNA and the PCR product resulted in the deletion of the *mdfA* sequence from nt −50 to 1327 (1,377-bp deletion) and its replacement with the *kan* gene. This was confirmed by two different PCRs. Deletion of *mdfA* in the transformants was first shown by PCR with primers MdfA3 (GCTGCGCTTTTATTAAACTCTGC) and MdfA4 (CCTGATCGCACAAATCATCA G), whose sequences correspond to sequences flanking the *mdfA* deletion and that resulted in a 1,227-bp fragment for the parental strains and a negative result when *mdfA* was deleted and replaced by the *kan* gene flanked by FLP. The third control PCR, with primers k2 (CGGTGCCCTGAAT GAACTGC) and kt (CGGCCACAGTCGATGAATCC), was used to detect the 471-bp *kan* fragment.

3. Results and Discussion

The effect of MdfA was confirmed by inactivating the *mdfA* gene located at different chromosomal loci in the strains studied. The antimicrobial susceptibilities of the parent and mutant strains are presented in Table 1. Both parent strains were resistant to ciprofloxacin (CIP) at MICs of 16 ug/ml and to NOR at MIC of 32 and 8 ug/ml, respectively. SF021787 and SF021895 were resistant to CIP at MICs of 12 ug/ml and to norprofloxacin (NOR) at MICs of 4 ug/ml regardless of the types of GyrA mutations, which suggests that without a functional MdfA. Compared MICs of the parent with △*mdfA* mutant strains, △*mdfA* mutant strain was more susceptible to CIP and NOR.

Interestingly, the resistance level to fluoroquinolone in the mutant strains harboring the △*mdfA* deletion was the same whether the strains carried three or four mutations in *gyrA,* it because, other efflux system, *acrAB* and *ndeh* contributed to resistance against fluoroquinolone.

The low-level changes in MICs of the △*mdfA* mutant suggest that *mdfA* contributed to the fluoroquinolone resistance in *S flexneri,* but it is not likely to be a major effector for higher resistance levels.

Acknowledgement

This study was supported by an intramural grant from the Korea National Institute of Health (2008-N00392-00).

References

1. Kim JY, Kim SH, Jeon SM, et al. Resistance to fluoroquinolone by the combination of target site mutations and enhanced expression of genes for efflux pumps in *Shigella flexneri* and *Shigella sonnei* strains isolated in Korea. Clin Microbiol Infect 2008 Aug;14(8):760−5.
2. Poole K. Efflux-mediated multiresistance in Gram-negative bacteria. Clin Microbiol Infect 2004 Jan;10(1):12−26.
3. Edgar R, Bibi E. MdfA, an *Escherichia coli* multidrug resistance protein with an extraordinally broad spectrum of drug recognition. J Bacteriol 1997 Apr;179(7):2274−80.
4. Yang S, Clayton SR, Zechiedrich EL. Relative contribution of the AcrAB, MdfA, and NorE efflux pumps to quinolone resistance in *Escherichia coli*. J Animicrobiol Chemother 2003 Mar;51(3): 545−56.
5. Datsenko KA, Wanner BL. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc Natl Acad Sci U S A 2000 Jun;97(12):6640−5.